INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 98/02550

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	"stratagene catalogue" January 1997 , STRATAGENE XP002085450 see page 274 - page 277 ---	1
Y	EP 0 726 310 A (GEN PROBE INC) 14 August 1996 see whole doc, esp. claims 13-27 ---	1-6
Y	SUGANUMA A. & CUPTA K.C.: "An evaluation of primer length on random-primed DNA synthesis for nucleic acid hybridization: longer is not better" ANALYTICAL BIOCHEMISTRY, vol. 224, - 1995 pages 605-608, XP002085448 cited in the application see the whole document --- -/--	1-6

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 November 1998	08/12/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Müller, F

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 98/02550

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication where appropriate of the relevant passages	Relevant to claim No
X	DE 195 03 685 A (INVITEK GMBH) 1 August 1996 see whole doc, esp. claims 1, 10,13; page 2,lin15 ff.	1-5
A	DAY I.N.M. ET AL.,: "Dried template DNA, Dride PCR oligonucleotides and mailing in 96-well:LDL receptor gene mutation screening" BIOTECHNIQUES, vol. 18, no. 6, - 1995 pages 981-984, XP002085449 see esp. page 982, 3.column ff.	1-6
A	WO 96 30544 A (WAKEFIELD ANDREW JEREMY) 3 October 1996 see whole doc. esp. claim 14	1-6
A	US 5 407 799 A (STUDIER F WILLIAM) 18 April 1995 see esp. claims (9,10)	1-6

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 98/02550

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0726310 A	14-08-1900	US 5556771 A	17-09-1996
		AU 4916796 A	27-08-1996
		CA 2210584 A	15-08-1996
		JP 10503383 T	31-03-1998
		WO 9624664 A	15-08-1996
		US 5614387 A	25-03-1997
		US 5834254 A	10-11-1998
DE 19503685 A	01-08-1996	NONE	
WO 9630544 A	03-10-1900	AU 5153196 A	16-10-1996
		CA 2216807 A	03-10-1996
		EP 0817864 A	14-01-1998
		GB 2300259 A	30-10-1996
US 5407799 A	18-04-1995	NONE	

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PCT GB98 0255

...TENT COOPERATION TRE... Y

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

PENNANT, Pyers
Stevens Hewlett & Perkins
1 Serjeants' Inn
Fleet Street
London EC4Y 1NT
ROYAUME-UNI

Date of mailing (day, month, year)
01 June 1999 (01.06.99)

Applicant's or agent's file reference
PP 1180

IMPORTANT NOTIFICATION

International application No.
PCT GB98 02550

International filing date (day, month, year)
21 August 1998 (21.08.98)

1. The following indications appeared on record concerning:

[ ] the applicant [ ] the inventor [X] the agent [ ] the common representative

Name and Address

PENNANT, Pyers
Stevens Hewlett & Perkins
1 Serjeants' Inn
Fleet Street
London EC4Y 1LL
United Kingdom

State of Nationality

State of Residence

Telephone No.
44 171 936 2499

Facsimile No.
44 171 936 2498

Teleprinter No.

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [ ] the name [X] the address [ ] the nationality [ ] the residence

Name and Address

PENNANT, Pyers
Stevens Hewlett & Perkins
1 Serjeants' Inn
Fleet Street
London EC4Y 1NT
United Kingdom

State of Nationality

State of Residence

Telephone No.
44 171 936 2499

Facsimile No.
44 171 936 2498

Teleprinter No.

3. ...

4. ...

[X] ...

[ ] ...

[X] ...

[ ] ...

[X] ...

[ ] ...

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Authorized officer

Dominique DELMAS

Telephone No.

. .ATENT COOPERATION TRE. Y

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
(Box PCT)
Crystal Plaza 2
Washington, DC 20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 05 May 1999 (05.05.99)	
International application No. PCT/GB98/02550	**Applicant's or agent's file reference** PP/1180
International filing date (day/month/year) 21 August 1998 (21.08.98)	**Priority date** (day/month/year) 22 August 1997 (22.08.97)
Applicant HOPKINS, Alison	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

13 March 1999 (13.03.99)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Fac. e No.: (41-22) 740.14.35

Authorized officer

Lazar Joseph Panakal

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference PP/1180	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/GB 98/ 02550	International filing date *(day/month/year)* 21/08/1998	(Earliest) Priority Date *(day/month/year)* 22/08/1997
Applicant NYCOMED AMERSHAM PLC et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [ ] **Certain claims were found unsearchable** (see Box I).

2. [ ] **Unity of invention is lacking** (see Box II).

3. [ ] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [ ] filed with the international application.
   - [ ] furnished by the applicant separately from the international application,
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] Transcribed by this Authority

4. With regard to the **title,**
   - [X] the text is approved as submitted by the applicant
   - [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**
   - [X] the text is approved as submitted by the applicant
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:
   Figure No. _____
   - [ ] as suggested by the applicant.
   - [ ] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.
   - [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 98/02550

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 November 1998	08/12/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Müller, F

1

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
X	DE 195 03 685 A (INVITEK GMBH) 1 August 1996 see whole doc, esp. claims 1, 10,13; page 2,lin15 ff.	1-5
A	DAY I.N.M. ET AL.,: "Dried template DNA, Dride PCR oligonucleotides and mailing in 96-well:LDL receptor gene mutation screening" BIOTECHNIQUES, vol. 18, no. 6, - 1995 pages 981-984, XP002085449 see esp. page 982, 3.column ff.	1-6
A	WO 96 30544 A (WAKEFIELD ANDREW JEREMY) 3 October 1996 see whole doc. esp. claim 14	1-6
A	US 5 407 799 A (STUDIER F WILLIAM) 18 April 1995 see esp. claims (9,10)	1-6

INTERNATIONAL SEARCH REPORT

[redacted]ation on patent family members

[redacted]ational Application No
[redacted]/GB 98/02550

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0726310 A	14-08-1900	US 5556771 A	17-09-1996
		AU 4916796 A	27-08-1996
		CA 2210584 A	15-08-1996
		JP 10503383 T	31-03-1998
		WO 9624664 A	15-08-1996
		US 5614387 A	25-03-1997
		US 5834254 A	10-11-1998
DE 19503685 A	01-08-1996	NONE	
WO 9630544 A	03-10-1900	AU 5153196 A	16-10-1996
		CA 2216807 A	03-10-1996
		EP 0817864 A	14-01-1998
		GB 2300259 A	30-10-1996
US 5407799 A	18-04-1995	NONE	

PATENT COOPERATION TREATY

REC'D 18 NOV 1999
WIPO PCT

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
PP/1180		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB98/02550	21/08/1998	22/08/1997
International Patent Classification (IPC) or national classification and IPC C12Q1/68		
Applicant NYCOMED AMERSHAM PLC et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   I ☒ Basis of the report
   II ☐ Priority
   III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   IV ☐ Lack of unity of invention
   V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   VI ☐ Certain documents cited
   VII ☒ Certain defects in the international application
   VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
13/03/1999	15. 11. 99
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Stricker, J-E Telephone No. +49 89 2399 8395

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB98/02550

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-11 as originally filed

Claims, No.:

1-6 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 2, 4, 6
	No:	Claims 1, 3, 5
Inventive step (IS)	Yes:	Claims none
	No:	Claims 1-6
Industrial applicability (IA)	Yes:	Claims 1-6
	No:	Claims none

2. Citations and explanations

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

Section V

Reference is made to the following documents:

D1: EP-A-0 531 027

D2: EP-A-0 726 310

D3: SUGANUMA A. & CUPTA K.C.: 'An evaluation of primer length on random-primed DNA synthesis for nucleic acid hybridization: longer is not better' ANALYTICAL BIOCHEMISTRY, vol. 224, - 1995 pages 605-608.

D4: DE-A-195 03 685

The documents D1 was not cited in the international search report (cf. PCT Guidelines, Chap. VI-7.24).

1. The document D1 shows the use of a labelling composition comprising a random mixture of oligonucleotide primers, preferably 6 to 9 nucleotides in length (cf. p.3, l.25-26 and claim 3). A dried labelling composition comprising a mixture of random 9-mer primers is disclosed p.4, l.11-27. A method of making labelled probes for a nucleic acid template by using the said 6 to 9-mer oligonucleotides is also disclosed (cf. p.3, "description of the invention").
   The applicant argue that a dried mixture of oligonucleotides which are 6 to 8 mers is not disclosed in D1, however this argument cannot be accepted for the following reasons.
   In D1, the description and example 1 are considered to represent connected information. Thus, when carrying out the method described in D1 (i.e. when producing the set of oligunucleotides which are 6 to 9 mers), the skilled person would automatically arrive at a dried composition falling within the scope of claims 1 and 3 of the present application and a method being the subject-matter of claim 5.

   Therefore, the subject-matter of claims 1, 3 and 5 does not meet the requirements of Art. 33(2) PCT.

2. The dependent claims 2, 4 and 6 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step (Art. 33(3) PCT), the reasons being as follows:

2.1 D1 discloses a kit for making labelled probes comprising the random mixture of oligonucleotides and a polymerase enzyme, a supply of nucleotides for chain extension and a buffer (p.3, l.51-55).
Adding a labelled nucleotide is merely one of several straightforward possibilities from which the skilled person would select, in accordance with circumstances, without the exercise of inventive skill (cf. D1, p.3, l.32 and p.4, l.37). Adding a dye to such a composition is common in the art (cf. the present application, p.2, l.16). The use of a stabiliser (e.g. trehalose) is also common for enzyme-containing dried compositions (cf. D2, p.5, l.33-39).
It would therefore be obvious to the person skilled in the art, to combine these features with corresponding effect, thereby arriving at a composition according to claim 2.

2.2 The feature of dependent claim 4 has already been employed for the same purpose for a similar composition (cf. the present application, p.2, l.15, or D2, p.5, l.55 and example 6 on p.15).

2.3 Making labelled probes using a random mixture of oligonucleotides at a final concentration falling within the range of 2-10 OD/ml is known from the prior art (e.g. in D3 which is also dealing with random mixtures of oligonucleotides and the use thereof, p.606, c.1, l.1-3, where 12.5 µl of reaction mixture contain 8.5 µl of previously made primer-template mixture containing 2.5 µg of random oligonucleotides, that means a final concentration of 200 µg/ml which should give an OD/ml of about 6.0, since 1.0 A_{260} unit ss DNA= 33 µg/ml).
Consequently, the subject-matter of claim 6 also lacks an inventive step.

3. The claimed subject-matter is considered to be novel over D4 (Art. 33(2) PCT) since a random mixture of oligonucleotides which are 6-mers to 8-mers is not disclosed therein.

Section VII

Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D1 is not mentioned in the description, nor is this document identified therein.

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12Q 1/68

A1

(11) International Publication Number: WO 99/10531

(43) International Publication Date: 4 March 1999 (04.03.99)

(21) International Application Number: PCT/GB98/02550

(22) International Filing Date: 21 August 1998 (21.08.98)

(30) Priority Data:
9717972.5 22 August 1997 (22.08.97) GB

(71) Applicant *(for all designated States except US)*: NYCOMED AMERSHAM PLC [GB/GB]; Amersham Place, Little Chalfont, Buckinghamshire HP7 9NA (GB).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: HOPKINS, Alison [GB/GB]; 39 Park Terrace, Tondu, Bridgend, Mid Glamorgan CF32 9HE (GB).

(74) Agents: PENNANT, Pyers et al.; Stevens Hewlett & Perkins, 1 Serjeants' Inn, Fleet Street, London EC4Y 1LL (GB).

(81) Designated States: CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: LABELLING COMPOSITION AND METHOD

(57) Abstract

A labelling composition comprises a random mixture of oligonucleotides which are 6–mers to 8–mers, said composition present in a dry state. A method of making labelled probes for a nucleic acid template comprises incubating the template under chain extension conditions with the labelling composition. The use of 6–mers to 8–mers reduces self–annealing, which is a problem with 9–mers in a dried state.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
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DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

LABELLING COMPOSITION AND METHOD

This invention concerns compositions comprising random mixtures of oligonucleotides and their use for labelling nucleic acids by a random prime method.

Feinberg and Vogelstein (1, 2) introduced the use of random sequence hexanucleotides to prime DNA synthesis on denatured template DNA at numerous sites along its length. The primer-template complex is a substrate for the "Klenow" fragment of DNA polymerase I. By replacing a non-radioactive nucleotide with the radiolabelled equivalent in the reaction mixture, newly synthesised DNA is made radioactive.

Very small amounts of input DNA can be labelled, enabling very high specific activity probes to be produced with relatively small quantities of added nucleotides. These radioactive labelled fragments can then be used as sensitive hybridisation probes for a wide range of filter based applications (3-6).

There are several labelling kits that are commercially available for the labelling of DNA by the random prime method. These include the Multiprime, Megaprime, Rediprime and Fluorescein Gene Images kits available from Amersham International plc. Ready-To-Go kits are available from Pharmacia and High Prime kits are available from Boehringer.

The Multiprime kit was introduced in the 1980s. It provides different tubes containing the different solutions that enable the user to make up labelling mixtures. One such tube contains a random mixture of 6-mer oligonucleotides, another the polymerase enzyme, and another the supply of nucleotides in the reaction buffer. All these separate solutions are stored frozen at -20°C. The purchaser thaws the different solutions, and adds precise quantities of each to the sample of denatured DNA that is

to be labelled, including a labelled nucleotide. This reaction is then usually incubated at 37°C at which temperature, oligonucleotide annealing and chain extension can occur. However, the reaction may also be incubated at lower temperatures such as an ambient room temperature of about 20°C.

The Megaprime kit was introduced commercially in the early 1990s. It is similar to the Multiprime kit, except that 9-mer oligonucleotides are used in place of 6-mers. The Megaprime kit has an advantage over the Multiprime kit, in that 9-mer oligonucleotides anneal more strongly (than do 6-mers) to a DNA target and form a hybrid having a higher melting temperature. Thus 9-mers achieve better and more rapid priming of a target then do 6-mers.

The Rediprime kit was introduced commercially in 1994. It comprises a mixture of 9-mer oligonucleotides with a polymerase enzyme and a supply of nucleotides. The mixture is supplied in a freeze-dried state. The freeze-dried mixture also contains a dye for easy visualisation. Dried kits for performing nucleic acid manipulation experiments were described by Ortlepp and McKay in EP 298 669 entitled "Performing nucleic acid reactions". The user reconstitutes the mixture by adding liquid containing the DNA template that is to be labelled, and then liquid containing the labelled nucleotide.

The Ready-To-Go kit was introduced during the 1990s. It is based on a random prime solution containing a random mixture of 9-mer or longer oligonucleotides, which solution is dried by a technique described in EP 383 569. A dye is not present. Like the Rediprime kit, the Ready-To-Go kit can be stored at +4°C or at ambient temperature. Promotional literature emphasises the speed of labelling, which results from the use of 9-mer oligonucleotides.

The High Prime kit is a wet kit containing a random mixture of oligonucleotides. The kit literature does not indicate what length of random oligonucleotides are used, but in the related document EP 649 909 A2, the

use of 6-mer, 9-mer, 12-mer and 15-mer is disclosed. No preferred length of random oligonucleotide is given. The solution is stablised by the use of glycerol and can be stored at between about -20°C and +4°C.

It can be seen that there has been a trend in commercial kits towards the use of longer oligonucleotides, particularly 9-mers or even longer. Going against this trend, it has been determined by Suganuma, A and Gupta, K C (7) that the use of long random primers, especially 9-mers or longer, reduces the priming efficiency of the random primer reaction. These authors worked on solutions which were used without being dried at any stage. The conclusions of these authors conflict with the findings of the present inventors; which findings are to the effect that, when experiments are done with solutions which are not dried, 9-mers provide more rapid and efficient labelling than do 6-mers, and do not give rise to any problem resulting from self-annealing or self-priming. To the best of applicants' knowledge, the conclusions reported by the authors of (7) have not caused the suppliers of random prime kits to use shorter oligonucleotides.

The present invention is based on the discovery that self-annealing occurs when random 9-mers are used in dried predispensed labelling kits, and that this limits their stability and shelf life. The self-annealing occurs during dispensing and storage when the random 9-mers anneal together to form primer-dimers or primer concatemers. These primer complexes become labelled during the normal labelling reaction, which concomitantly reduces the amount of label that is incorporated into copies of the template that are being synthesised during the reaction. Shorter oligonucleotides are not subject to this problem. The problem is specific to 9-mers (and longer oligonucleotides) used in dried kits.

The invention provides a labelling composition comprising a random mixture of oligonucleotides which are 6-mers to 8-mers, said composition present in a dry state. Preferably the composition also contains at least one of: a polymerase enzyme; a supply of nucleotides for

chain extension; a labelled nucleotide; a dye; a stabiliser; and a buffer.

As the experimental data below shows, 5-mer oligonucleotides are too short to be useful in dried kits. As the length of the oligonucleotides increases from 6-mers to 9-mers, there is a concomitant increase in the aforementioned self-priming problem. On the other hand, longer oligonucleotides anneal more rapidly and strongly to templates than do shorter ones. Taking into account both these factors, applicants believe that 6-mer oligonucleotides are more preferable than 7-mers which in turn are more preferable than 8-mers.

The random mixture of oligonucleotides is present in a dry state. Various drying techniques are possible, including that described in EP 383 569, and also freeze-drying or lyophilisation which is preferred.

It is possible to use any DNA polymerase enzyme in the labelling reaction, for example Klenow, exonuclease free klenow, DNA polymerase I, T7 DNA polymerase, Sequenase™, Thermosequenase™, so long as the reaction buffer conditions are suitable for the specific enzyme being used.

All four of the nucleotides are preferably present in the composition, whether labelled or unlabelled, and the relative molar concentrations may be adjusted to improve the efficiency of labelling. Also when a labelled nucleotide is present, the equivalent unlabelled nucleotide may also be present to improve the efficiency of labelling, or to control the specific activity of the DNA that is being produced from the labelling reaction.

These compositions enable a DNA template to be used to produce copies which are labelled radioactively, for example, by using either phosphate labelled with P-32 or S-35, or by using H-3 or C-14 base labelled nucleotides. Alternatively non-radioactive labels may be used, for example, fluorescein, biotin, digoxigenin, rhodamine and cyanine dyes, may be incorporated when, for example, covalently linked to the base moiety of the nucleotide.

Any stabiliser may be present to protect the activity of the enzyme, for example, trehalose, sucrose, BSA, gelatin. A dye may also be present to allow the dried pellet to be visualised, before use, and to assist in determining that mixing is thorough.

The invention also includes a method of making labelled probes for a nucleic acid template, which method comprises incubating the nucleic acid template under chain extension conditions with the labelling composition as herein described. Preferably the template is DNA. The inventor has found that random 6-mers can give fast labelling kinetics (10 minutes labelling time) by being present at high concentration in the reaction mixture. A preferred concentration is 2-10 O.D./ml in the final reaction with about 5 O.D./ml being most preferable. A probe labelled in this manner is suitable for use in a Southern hybridisation.

All the results shown in the examples show labelling with dCTP-^{32}P, but this is only as a means to show, and quantitate the amount of self-priming that occurred in each reaction. The reactions are able to label DNA with other labels, both radioactive and non-radioactive, as indicated elsewhere in this specification.

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Example 1. Manufacture of lyophilised reactions with different random primer lengths:

All primers were diluted to 50 O.D./ml in water. The number of enzyme units was the same in each reaction (7 units).

The amount of each component solution is as follows for a 6 ml scale.

	5 mer reaction mix	6 mer reaction mix	7 mer reaction mix	8 mer reaction mix	9 mer reaction mix
Nucleotide buffer	1.998 ml	1.998 ml	1.998 ml	1.998 ml	1.998 ml
Exo-free Klenow (12 µl) 100 units/µl	1200 units	1200 units	1200 units	1200 units	1200 units
Dilution Buffer	28 µl	28 µl	28 µl	28 µl	28 µl
5 mer primer	1.0 ml				
6 mer primer		1.0 ml			
7 mer primer			1.0 ml		
8 mer primer				1.0 ml	
9 mer primer					1.0 ml
20% Trehalose	1.5 ml	1.5 ml	1.5 ml	1.5 ml	1.5 ml
0.2 mg/ml Xylene Cyanol	0.198 ml	0.198 ml	0.198 ml	0.198 ml	0.198 ml
PF Water	1.264 ml	1.264 ml	1.264 ml	1.264 ml	1.264 ml
Total Volume	6 ml	6 ml	6 ml	6 ml	6 ml

Each reaction mix was dispensed into tubes in 35 µl aliquots, and were freeze dried.

Methods:

1. Nucleotide buffer: Labelling buffer from Nick Translation kit (N5000/N5500 Amersham International plc).

2. Dilution buffer: Storage buffer for enzyme dilution.

3. Labelling Method: Tubes of DNA for labelling were made up as follows:

5 μl λ HindIII DNA at 5 ng/μl in TE buffer.

40 μl water.

Placed all tubes in a boiling water bath (95 to 100°C) for 5 minutes,

placed all tubes on ice for 5 minutes, centrifuged briefly,

then added the denatured DNA solutions to the respective dried reaction tube samples

added 5 μl Redivue™ dCTP (α^{32}P) (Product Code AA0005: Amersham International plc) (50 μl total reaction volume).

Incubated all reactions for 10 minutes at 37°C.

Spotted 2 μl samples out onto PEI-cellulose tlc plates,

Ran plates in 1.25 M KH_2PO_4 pH 3.4.

Analysed plates on plate scanner, to measure the %incorporation, %self-priming and %dCTP present at the end of each reaction.

The %self-priming is defined as the % of the total radioactive counts that are situated between the incorporated counts and the counts due to the unincorporated dCTP-^{32}P.

λ HindIII DNA Labelling with dCTP-^{32}P (Week 1 Test)

Tube	Primer Type	Tube-1			Tube-2		
		% Incorp	% Self-Prime	% dCTP	% Incorp	% Self-Prime	% dCTP
1, 2	5 mers	62.7	7.9	23.0	54.8	7.7	30.9
3, 4	6 mers	79.9	11.2	2.7	82.1	10.8	2.2
5, 6	7 mers	73.5	17.5	2.8	74.3	15.1	3.7
7, 8	8 mers	68.3	19.1	3.2	65.6	20.4	3.6
9, 10	9 mers	64.9	23.7	3.1	61.5	27.2	3.1

The column headed "% Incorp" shows the percentage of dCTP-^{32}P incorporated as a chain extension product of a primer-λHind III DNA hybrid. The column headed "% Self-Prime" shows the percentage of dCTP-^{32}P incorporated in a complex involving only primers. The column headed "% dCTP" shows the percent of unincorporated dCTP-^{32}P. The % dCTP figures were unacceptably high when 5-mer oligonucleotides were used, but were acceptable for 6-mers to 9-mers. Within this range, the % Incorp figures decrease as the oligonucleotide length increases from 6 to 9.

Example 2. Long term stability comparison of dried reactions, nonamers compared with hexamers, 3.5 units of Exo-free Klenow per reaction:

The samples were made up as shown in Example 1, but 6 μl of Exo-free Klenow was used.

DNA Labelling with dCTP-^{32}P, results are the averages of the three reactions

	Nonamers			Hexamers		
Week	% Incorp	% Self-Prime	% dCTP	% Incorp	% Self-Prime	% dCTP
3	61.9	17.5	6.2	69.6	9.7	6.3
6	71.4	18.0	4.3	80.8	8.4	4.7
10	65.8	20.2	6.4	75.0	11.9	6.9
16	66.5	16.5	8.0	73.4	11.1	6.5
21	78.3	10.8	3.0	84.3	5.8	2.4
25	42.7	11.6	40.4	55.1	5.4	35.1

As these figures show, the % incorporation of dCTP-^{32}P when using 9-mers was initially lower than when using 6-mers and remained lower on storage of the compositions for up to 25 weeks.

Example 3:

Using dried reactions as shown in Example 1, the primer was replaced with water for the reaction drying, and was added later as a separate solution, when the reactions were being used. All reactions were incubated for 10 minutes, and then sampled to measure the % incorporation.

Primer Concentration in reaction O.D./ml	% Incorporation (hexamer primers) Average of two reactions	% Incorporation (nonamer primers) one reaction
6.0	78.3	
5.0	83.2	81.0
4.0	67.7	65.6
2.0	51.5	67.0
1.0	45.1	60.2

It can be seen from these results that the same primer concentration (O.D./ml) is required to achieve the same reaction kinetics, i.e. the same % incorporation in 10 minutes with different random primer lengths. This shows that the molar concentration needs to increase as the primer length is reduced.

Although the above results were obtained using wet reagents, the conclusion would apply also when dry primers are used.

Example 4:

Densitometer results of Southern hybridisations

25ng labelling reactions were carried out using the Megaprime Labelling Kit RPN 1606 (Amersham International plc) or using labelled probes from dried nonamer or hexamer labelling reactions made as described above in other examples. Southern blots were hybridised for 2 hours at 65°C with the labelled probe under standard conditions and then washed in 2 x SSC, 0.1% SDS, 20 minutes at room temperature, followed by two washes in 0.5 x SSC, 0.1% SDS, for 5 minutes 65°C. The dried blots were detected on X-ray film with 2 intensifying screens and place into a -70°C freezer, for 16 hours. After the film was developed using a film processor it was scanned using a densitometer, then the results were analysed using ImageQuant software.

Kit	Time of test after manufacture	Target	%band intensity of Southern hybridisation cf Megaprime control
9mers	1 week	0.25pg	42.23
9mers	1 week	0.5pg	40.12
9mers	1 week	1.0pg	38.93
6mers	1 week	0.25pg	97.09
6mers	1 week	0.5pg	95.02
6mers	1 week	1.0pg	94.33
6mers	37 weeks	0.25pg	74.58
6mers	37 weeks	0.5pg	80.91
6mers	37 weeks	1.0pg	81.17

Conclusions:

The hexamers used in a dried labelling reaction generate labelled probes which gave a much stronger band intensity than when nonamers are used, not only when tested initially after 1 week, but even after an extended period of storage (37 weeks at room temperature).

CLAIMS

1. A labelling composition comprising a random mixture of oligonucleotides which are 6-mers to 8-mers, said composition present in a dry state.

2. A labelling composition as claimed in claim 1, wherein the composition also contains at least one of: a polymerase enzyme; a supply of nucleotides for chain extension; a labelled nucleotide; a dye; a stabiliser; and a buffer.

3. A labelling composition as claimed in claim 1 or claim 2, wherein the random mixture is of 6-mer oligonucleotides.

4. A labelling composition as claimed in any one of claims 1 to 3, wherein the composition is present in a freeze-dried state.

5. A method of making labelled probes for a nucleic acid template, which method comprises incubating the nucleic acid template under chain extension conditions with the labelling composition of any one of claims 1 to 4.

6. A method as claimed in claim 5, wherein the random mixture of oligonucleotides is present at a concentration of 2-10 O.D./ml.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 98/02550

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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X	"stratagene catalogue" January 1997 , STRATAGENE XP002085450 see page 274 - page 277 ---	1
Y	EP 0 726 310 A (GEN PROBE INC) 14 August 1996 see whole doc, esp. claims 13-27 ---	1-6
Y	SUGANUMA A. & CUPTA K.C.: "An evaluation of primer length on random-primed DNA synthesis for nucleic acid hybridization: longer is not better" ANALYTICAL BIOCHEMISTRY, vol. 224, - 1995 pages 605-608, XP002085448 cited in the application see the whole document --- -/--	1-6

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
24 November 1998	08/12/1998

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 98/02550

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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A	DAY I.N.M. ET AL.,: "Dried template DNA, Dride PCR oligonucleotides and mailing in 96-well:LDL receptor gene mutation screening" BIOTECHNIQUES, vol. 18, no. 6, - 1995 pages 981-984, XP002085449 see esp. page 982, 3.column ff. ---	1-6
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A	US 5 407 799 A (STUDIER F WILLIAM) 18 April 1995 see esp. claims (9,10) -----	1-6

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Information on patent family members

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